Earliest Evidence of Preclinical Diabetic Retinopathy Revealed Using Optical Coherence Tomography Angiography Perfused Capillary Density

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• PURPOSE: To compare perfused capillary density (PCD) in diabetic patients and healthy controls using optical coherence tomography angiography (OCTA).

• METHODS: Forty controls, 36 diabetic subjects without clinical retinopathy (NoDR), 38 with nonproliferative retinopathy (NPDR), and 38 with proliferative retinopathy (PDR) were imaged using spectral-domain optical coherence tomography. A 3 × 3-mm full-thickness parafoveal OCTA scan was obtained from each participant. Following manual delineation of the foveal avascular zone (FAZ), FAZ area, perimeter, and acircularity index were determined. Seven consecutive equidistant 200-μm-wide annular segments were drawn at increasing eccentricities from the FAZ margin. Annular PCD (%) was defined as perfused capillary area divided by the corresponding annulus area after subtraction of noncapillary blood vessel areas. Nonparametric Kruskal-Wallis testing with Bonferroni correction was performed in pairwise comparisons of group PCD values.

• RESULTS: The NoDR group demonstrated consistently higher PCD compared to the control group in all 7 annuli, reaching statistical significance (36.6% ± 3.30% vs 33.6% ± 3.98%, $P = .034$) at the innermost annulus (FAZ margin to 200 μm out). The NPDR and PDR groups demonstrated progressively decreasing PCD. Differences in FAZ metrics between the NoDR and control groups did not reach statistical significance.

• CONCLUSIONS: Relative to healthy controls, increased PCD values in the NoDR group likely represent an autoregulatory response to increased metabolic demand, while the decrease in PCD that follows in NPDR and PDR results largely from an incremental loss of capillary segments. These findings, consistent with previous studies, demonstrate the potential of OCTA as a clinical tool for earlier objective detection of preclinical diabetic retinopathy. NOTE: Publication of this article is sponsored by the American Ophthalmological Society. (Am J Ophthalmol 2019;203:103–115. © 2019 Published by Elsevier Inc.)

THE GLOBAL PREVALENCE OF DIABETES MELLITUS HAS reached epidemic proportions, estimated to have affected 415 million people in 2015, and is expected to affect 642 million by 2040.[1] Diabetic retinopathy is the most common complication of diabetic microvascular disease, and it is the leading cause of vision loss in adulthood.[2] Additionally, diabetes is a major cause of life-threatening complications such as end-stage renal disease,[3] myocardial infarction, stroke, and peripheral vascular disease.[4,5]

Many insights into the microvascular changes that occur from long-term exposure to hyperglycemia have been gained from taking advantage of the transparency of ocular structures and examining the living retina.[6] The visualization of retinal vascular lesions has helped scientists and clinicians better understand the natural course of the disease.[7] Nonproliferative diabetic retinopathy (NPDR) has been used as an umbrella term for the clinically identifiable biomarkers of diabetic microvascular disease in the retina prior to the development of more vision-threatening proliferative changes.[8] The earliest clinically observable signs of NPDR include microaneurysms, capillary nonperfusion, and dot-and-blot intraretinal hemorrhages.[9]

The ability to monitor tissue response to treatment through serial examinations of the retina has been used to guide management goals of hyperglycemia with systemic medicines such as oral hypoglycemics and insulin in attempts to lower the risk of diabetic damage to the eye as well as other microvascular and macrovascular end-organ complications.[10,11] This approach may be more sensitive than the reverse, since in some patients structural changes occur despite apparent good metabolic control.[12] The relative success achieved with current retinal screening and treatment

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protocols[13] has encouraged further research to identify earlier preclinical biomarkers of microvascular abnormality in the diabetic retina. The hope is that these markers will allow clinicians to diagnose disease and stratify patients according to their risk for end-organ complications earlier. This is important, since earlier treatment is associated with better outcomes.[14] These markers would potentially allow clinicians to monitor tissue response to current and emerging systemic and ocular treatment modalities in a more sensitive and individualized manner. Novel preclinical biomarkers could also shed additional light on the pathophysiology of diabetic microvascular disease and retinopathy.

Advances in retinal imaging and image processing techniques have allowed researchers to detect earlier signs of disease preceding classic NPDR, including structural markers such as arteriolar and venular caliber changes, tortuosity progression, and foveal avascular zone (FAZ) size and shape alterations, as well as functional markers such as vasodilatory response, blood flow, and oxygen saturation variances.[15] A significant advancement in retinal microvascular imaging, adaptive optics scanning light ophthalmoscopy (AOSLO), was the first technology to reveal the delicate structure of capillaries at a much higher resolution than conventional fluorescein angiography (FA) in a variety of modalities, including confocal and nonconfocal techniques.[16-20] Advancing the lateral image resolution on clinical subjects by at least 1 order of magnitude (from 20 μm to 2 μm), AOSLO brought vascular wall components, microaneurysms, and single capillary segment details into focus.[16,17,21-23] Its major limitation has been the time-consuming off-line processing effort required for outputting results, which may take several hours to several days. The vascular details revealed by this laboratory technique have raised awareness of the need for an imaging technology that is capable of imaging capillary beds in a clinically relevant time scale.

Optical coherence tomography angiography (OCTA), a more recent development that takes advantage of higher-speed OCT and sophisticated image processing algorithms,[24-26] provides comparable quantitative and qualitative retinal microvascular details to AOSLO-FA,[27] and does so in 3 dimensions and with an immediacy that fosters clinical relevance. The clinical impact of this new imaging modality is still unfolding, since it provides access to functional data concealed within the structural features of the image. Some of the novel biomarkers that have been proposed include detection of nonperfused capillaries,[28] changes in FAZ metrics,[29-31] changes in perifoveal intercapillary area,[32] and functional markers such as adjusted flow index.[33]

In our laboratory, we initially developed a number of qualitative tools, including perfused vessel density calculations and vessel density color maps based on skeletonized AOSLO FA perfusion maps, to study the retinal microvasculature in healthy eyes and in eyes with venous occlusive disease.[34,35] A version of this method was applied to OCTA through a collaboration between members of our team and the software engineers at Optovue, enabling Agemy and associates to demonstrate that it could objectively categorize levels of progressive diabetic retinopathy consistent with expert reader interpretation.[36] Other similar techniques of OCTA perfusion density mapping have been developed and used to suggest that perfused vessel density is decreased in diabetic eyes without retinopathy.[37-42] These studies employed different variations of perfused vessel density, largely relying upon the percent area occupied by perfused vessels divided by total sampled area, and included noncapillary blood vessels in their analysis.

The purpose of this study was to assess parafoveal perfused capillary density (PCD) using OCTA, with an analysis that specifically excludes noncapillary blood vessels, in patients with diabetes but no clinical evidence of retinopathy and patients with various clinical stages of diabetic retinopathy, compared to healthy controls. Our findings suggest that OCTA PCD, after excluding noncapillary blood vessels, may be a novel and an even more sensitive biomarker for detecting the earliest diabetic changes in patients with no other evidence of clinically observable retinopathy, as well as objectively monitoring the clinical course of the disease.

METHODS

• **STUDY POPULATION:** Written consent was obtained from all participants prior to imaging. The protocol conformed to the tenets of the Declaration of Helsinki, was HIPAA compliant, and was prospectively approved by the Institutional Review Boards of the New York Eye and Ear Infirmary of Mount Sinai and the Medical College of Wisconsin. Forty controls with no history of intraocular pathology or major systemic vascular disease were recruited for this study. Seventy-eight diabetic patients with and without retinopathy were also recruited. Diabetic patients were divided into 3 groups: 36 patients with no clinically observable diabetic retinopathy (NoDR), 38 patients with NPDR, and 38 patients with proliferative diabetic retinopathy (PDR). Seventeen of the controls were recruited at the Medical College of Wisconsin, with the rest of the study participants being recruited at the New York Eye and Ear Infirmary of Mount Sinai. All diabetic patients underwent comprehensive ophthalmic examination including slit-lamp examination and dilated funduscopy. Inclusion criteria for both healthy controls and diabetic patients were as follows: normal anterior segment and clear media. Diabetic patients without clinical retinopathy were examined by a retina specialist to rule out any presence of microaneurysms, hemorrhages, or ophthalmoscopically detectable evidence of capillary nonperfusion. Diabetic patients had a BCVA of 20/80 or better. Exclusion criteria included nuclear, cortical, or posterior subcapsular cataracts ≥ grade 3 according to the Lens Opacity Classification

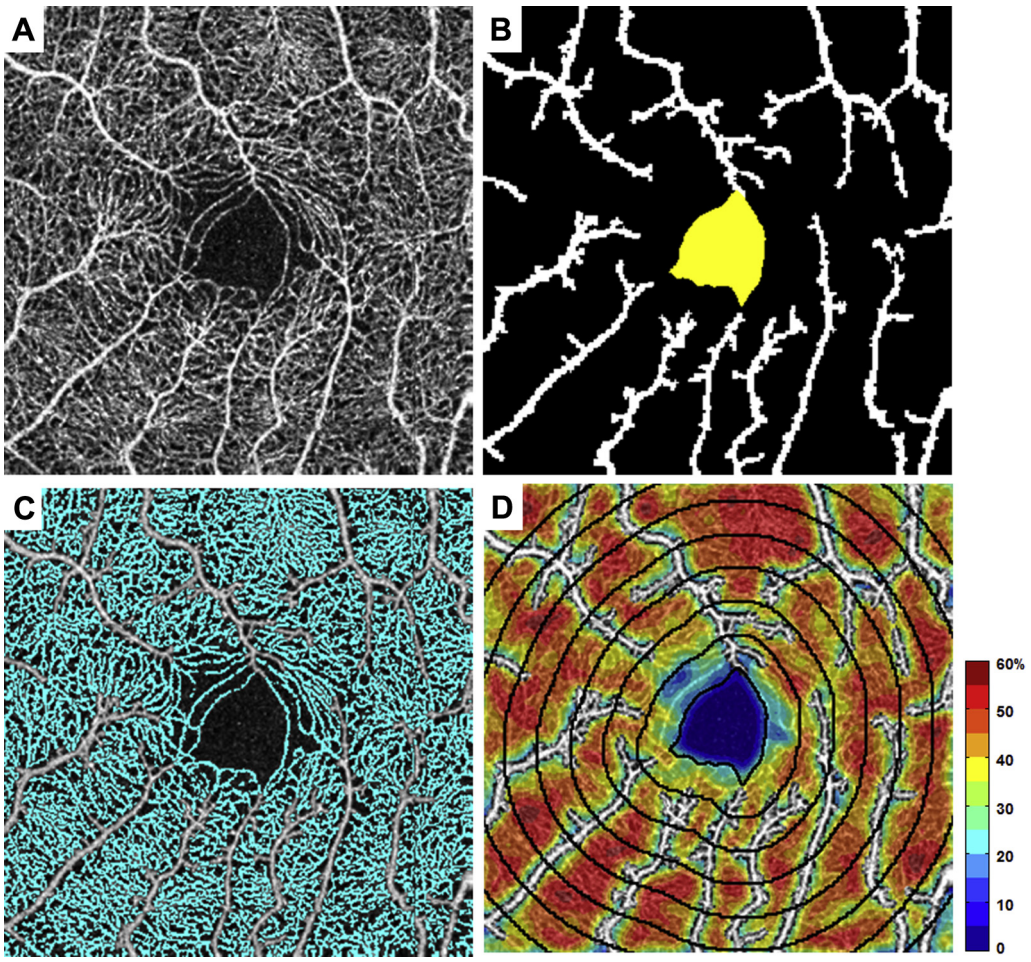

FIGURE 1. Optical coherence tomography angiography (OCTA) image processing procedure. (A) Contrast-stretched full vascular slab OCTA. (B) Manual segmentation of foveal avascular zone (in yellow) and automatic segmentation of noncapillary blood vessels using global thresholding (in white). (C) Automatic segmentation of capillaries (in cyan) after the removal of noncapillary blood vessels. (D) Overlay of perfused capillary density map with 7 consecutive 200-μm equidistant annuli starting from the foveal avascular zone margin going outward.

System III[43]; active macular edema, and past ocular surgery including cataract and refractive surgery. One eye from each participant was included for imaging and data analysis.

• **OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY IMAGE ACQUISITION:** Using a commercial spectral-domain OCT system (Avanti RTVue-XR; AngioVue version 2016.2.0.16 and 2016.2.0.35; Optovue, Fremont, California, USA), a 3 × 3-mm macular OCTA scan was obtained in each participant. The scans were segmented to isolate the full vascular slab between the inner limiting membrane and 70 -μm below the posterior boundary of the inner plexiform layer, including both superficial and deep capillary layers, and noncapillary blood vessels. This OCT system has an A-scan rate of 70 000 scans per second using a light source centered at 840 nm and a bandwidth of 45 nm. Each OCTA scan was composed of 2 volumetric raster scans (1 horizontal and 1 vertical) with 304 A-scans per B-scan and 608 total B-scans per volumetric raster scan. OCTA images were created using the split-spectrum amplitude decorrelation angiography (SSADA) algorithm incorporated into the device.[44] Individual axial length measurements were obtained using an IOLMaster (Carl Zeiss Meditec, Inc, Dublin, California, USA) to correct the retinal magnification of each OCTA image.

• **OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY IMAGE PROCESSING:** The OCTA image processing and analysis was done in Matlab (The MathWorks, Inc, Natick, Massachusetts, USA) and was similar to our previously published studies (Figure 1).[45] First, each grayscale full vascular slab OCTA image (304 × 304 pixels) was resized by a factor of 6 (1824 × 1824 pixels) (Figure 1A). Then, the FAZ was manually delineated, creating an FAZ mask using Adobe Photoshop CS6 (Adobe System Inc, San Jose, California, USA) (Figure 1B, yellow area). FAZ

metrics including area, perimeter, and acircularity index were computed based on the FAZ mask as previously described.[31] Global thresholding was then applied to the contrast-stretched full vascular slab OCTA by replacing all pixels with intensity greater than 0.7 with the value 1 (white) and the remaining pixels with the value 0 (black) (Figure 1B, white area). This binary image was used as a mask for the removal of the area associated with the noncapillary blood vessels on the full vascular slab OCTA. After the removal of noncapillary blood vessels, local thresholding was performed for the segmentation of parafoveal perfused capillaries (Figure 1C). For qualitative assessment, a color-coded PCD map was created (Figure 1D). On the PCD map, noncapillary blood vessels appear in white, as they were excluded from the computation.

To ensure that the same region of interest was compared across all full vascular slab OCTA images with varying FAZ sizes and shapes, distance transformation of the FAZ mask was performed to create 7 consecutive equidistant annuli of increasing retinal eccentricity from the FAZ margin, each with a width of 200 μm (Figure 1D). The innermost annulus included the area of the retina from the FAZ margin (0 μm) outward to 200 μm from the FAZ margin. The outermost annulus represented retinal distance from 1200 to 1400 μm from the FAZ margin.

For quantitative assessment, PCD and noncapillary blood vessel density (%) for each annulus were then computed as described below:

$$\text{Perfused capillary density, \%} = \frac{\text{Perfused capillary area}}{\text{Annulus area} - \text{Noncapillary blood vessel area}} \times 100\%$$

$$\text{Noncapillary blood vessel density, \%} = \frac{\text{Noncapillary blood vessel area}}{\text{Annulus area}} \times 100\%$$

• **STATISTICAL ANALYSIS:** Statistical analysis was performed using commercial statistical software (SPSS, IBM Analytics; IBM Corporation, Armonk, New York, USA). Sex differences in FAZ metrics, PCD, and noncapillary blood vessel density were assessed using Mann-Whitney *U* tests. Nonparametric Kruskal-Wallis tests with Bonferonni correction for pairwise comparison were used to assess age and axial length differences between study groups.

Two-way analysis of variance (ANOVA) with post hoc Bonferroni correction was used to assess the effect of study group and annulus on PCD. Nonparametric Kruskal-Wallis testing with Bonferroni correction for pairwise comparison was used to assess differences between study groups in FAZ metrics and PCD at each annulus. A parallel analysis was performed on noncapillary blood vessel density values to ensure that there was no confounding effect of noncapillary blood vessel changes on PCD results. *P* values less than .05 were considered statistically significant. The diagnostic capability of PCD at the 200-μm annulus to differentiate between control and NoDR eyes was assessed using the area under the receiver operating characteristic curve (AUROC), sensitivity at 95% specificity, and specificity at 95% sensitivity.

TABLE 1. Demographic Data of the 4 Study Groups

	Control	NoDR	NPDR	PDR
No. of participants	40	36	38	38
Male/female	20/20	18/18	18/20	19/19
Mean age ± SD, years	59.7 ± 7.89	57.69 ± 9.59	58.34 ± 6.81	55.58 ± 5.18
DM type I/II	NA	2/34	4/34	3/36

DM = diabetes mellitus; NoDR = no clinically observable diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

RESULTS

ONE HUNDRED AND FIFTY-TWO EYES OF 152 PARTICIPANTS (75 male, 77 female) were analyzed. Participant demographic information is listed in Table 1. Although female participants showed significantly larger FAZ areas (female: 0.38 ± 0.15 mm^2 vs male: 0.32 ± 0.12 mm^2; $P = .003$) and smaller FAZ acircularity indexes (female: 1.39 ± 0.24 vs male: 1.48 ± 0.31; $P = .02$) compared to male participants, no sex difference was observed in FAZ perimeter. No sex difference was observed in PCD and noncapillary blood vessel density at any annulus. There were no significant differences in age or axial length between the 4 study groups.

• **FOVEAL AVASCULAR ZONE METRICS:** Box plots of FAZ area, perimeter, and acircularity index are shown in Figure 2, with brackets indicating statistical significance between groups. The mean and standard deviation [SD] values of all 3 FAZ metrics for the 4 study groups are shown in Table 2. FAZ area was significantly greater in the PDR group compared to the control and NoDR groups.

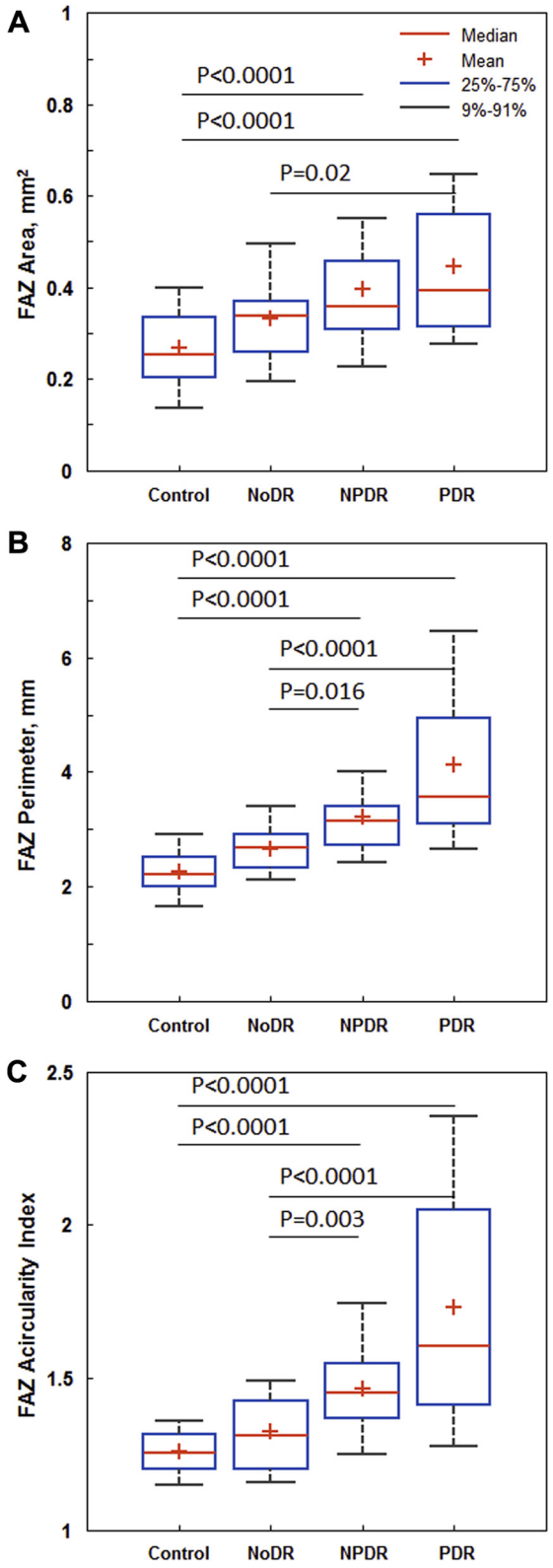

FIGURE 2. Box plots of foveal avascular zone (FAZ) metrics. (A) FAZ area. (B) FAZ perimeter. (C) FAZ acircularity index. Brackets indicate statistically significant differences between corresponding study groups. NoDR = no clinically observable diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

Similarly, FAZ perimeter and acircularity index were significantly greater in the NPDR and PDR groups compared to the control and NoDR groups. Notably, no statistically significant difference was detected between the control and the NoDR groups in any of the FAZ metrics.

• **PERFUSED CAPILLARY DENSITY:** PCD group mean values per annulus are shown in Figure 3. PCD was highest in the NoDR group, followed by the control, NPDR, and PDR groups. Mean PCD values and 95% confidence intervals for each annulus are displayed in Table 3. Using 2-way ANOVA, a significant main effect on PCD was found between the study groups ($P = 4.81 \times 10^{-118}$). Post hoc analyses indicated that all 4 study groups differed significantly ($P < .0001$). Importantly, PCD was significantly higher in the NoDR group compared to the other 3 study groups. As expected, 2-way ANOVA showed a significant main effect of annulus on PCD values ($P < 2.14 \times 10^{-53}$), with post hoc analyses indicating that PCD measured at the 200-μm annulus was significantly lower than that of the other 6 annuli ($P < .0001$). There was no significant interaction between study group and annulus ($P = .27$).

While the NoDR group showed consistently higher PCD compared to the control group at all annuli, nonparametric Kruskal-Wallis tests indicated that only the 200-μm annulus reached statistical significance (NoDR 36.6% ± 3.30% vs control 33.6% ± 3.98%, $P = .034$; Figure 4A). In discriminating between the control and NoDR eyes, PCD measured at the 200-μm annulus showed an AUROC of 0.713 (95% confidence interval: 0.598-0.827), with sensitivity at 95% specificity of 66.7% (95% confidence interval: 0-90%), and specificity at 95% sensitivity of 73.5% (95% confidence interval: 38.2%-91.2%). Box plots of PCD measured at the 200-μm, 800-μm, and 1400-μm annuli are shown in Figure 4, with brackets indicating statistically significant differences between corresponding study groups.

Color-coded PCD maps of 4 representative individual eyes, 1 from each of the 4 study groups, are shown in Figure 5, demonstrating qualitative and quantitative comparisons. The first column indicates the contrast-stretched full vascular slab OCTA image from each group. The second column shows the overlay of capillary segmentation (in cyan) over the full vascular slab OCTA image after removal of noncapillary blood vessels. Color-coded PCD maps are shown in the last column with the 200-μm annulus delineated with a black border for comparison.

• **NONCAPILLARY BLOOD VESSEL DENSITY:** Noncapillary blood vessel density group mean values per annulus are shown in Figure 3. Box plots of noncapillary blood vessel density measured at the 200-μm, 800-μm, and 1400-μm annuli are shown in Figure 4, with brackets indicating statistically significant differences between corresponding study groups. Nonparametric Kruskal-Wallis tests

TABLE 2. Foveal Avascular Zone Metrics of the 4 Study Groups

	Control	NoDR	NPDR	PDR
FAZ area Mean ± SD, mm^2	0.27 ± 0.09	0.33 ± 0.10	0.40 ± 0.16	0.45 ± 0.19
FAZ perimeter Mean ± SD, mm	2.27 ± 0.44	2.66 ± 0.51	3.22 ± 0.79	4.12 ± 1.72
FAZ acircularity index Mean ± SD	1.26 ± 0.08	1.32 ± 0.15	1.46 ± 0.16	1.73 ± 0.42

FAZ = foveal avascular zone; NoDR = no clinically observable diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

indicated that only the 200-μm, 600-μm, and 800-μm annuli showed statistically significant differences across groups. At the 200-μm annulus, the control group showed a significantly lower noncapillary blood vessel density compared to the PDR group (Figure 4B). At the 600-μm and 800-μm annuli, the NoDR group showed a significantly higher noncapillary blood vessel density compared to the PDR group (Figure 4D). No significant difference was found in noncapillary blood vessel density between the control and NoDR groups across any annulus.

DISCUSSION

IN THIS STUDY, WE FOUND THAT THE NODR GROUP DEMONstrated a consistently higher PCD compared to the healthy control group in all 7 annuli, with the first annulus (FAZ margin to 200 μm from the FAZ margin) reaching statistical significance. Notably, PCD was more sensitive than FAZ metrics for detecting a difference between these 2 groups. In agreement with previously reported data, NPDR and PDR groups exhibited progressively decreasing perfused vessel density.[36,46] Additionally, we found no significant difference in noncapillary blood vessel density between the healthy controls and NoDR patients across all annuli, which implies the capillaries as the source of the difference between these 2 groups. This shift from PCD elevation to progressive decline suggests a meaningful inflection or "tipping point," which may have value as a preclinical biomarker of diabetic microvascular disease, signaling that normal compensatory responses have become overwhelmed just prior to the appearance of clinically visible lesions. Exceeding this threshold is important not only for vision loss, but for other systemic complications as well. Retinal capillary beds are particularly vulnerable, showing signs of diabetic microvascular disease early owing to their high metabolic demands. Though less easily recognized, other end-organs are likely not far behind.

Using OCTA, a few recent studies have reported a decrease in macular perfused vessel density in type 1 and type 2 diabetics without diabetic retinopathy compared to normal controls.[37-42] The major difference between those studies and ours was the way we processed the OCTA to selectively isolate the capillary change. In our study, PCD was specifically defined as the percentage of capillary area divided by the corresponding annulus area once noncapillary blood vessel areas had been subtracted. The previous studies cited included noncapillary blood vessels in the density analysis. We strongly believe that this lack of selectivity reduces the sensitivity of the analysis and misses the peak in PCD prior to its progressive decline. Structural

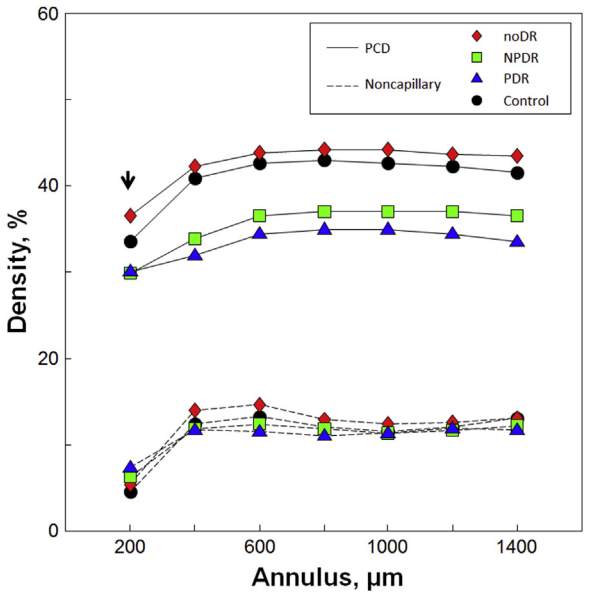

FIGURE 3. Group mean PCD and noncapillary blood vessel density values measured at each annulus. The NoDR group consistently showed higher PCD values compared to the other groups, including the control group. Only the 200-μm annulus showed a statistically significant increase in PCD in the NoDR group compared to the control group (arrow, $P = .034$). NoDR = no clinically observable diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

TABLE 3. Group Mean PCD Values and 95% Confidence Intervals Measured at Each Annulus

Annulus	Mean PCD % (95% Confidence Interval)			
	Control	NoDR	NPDR	PDR
200-μm	33.6 (32.4-34.9)	36.6 (35.5-37.8)	29.8 (28.2-31.4)	30.1 (28.8-31.4)
400-μm	41.0 (39.9-42.1)	42.4 (41.5-43.2)	33.9 (32.3-35.4)	32.1 (30.5-33.7)
600-μm	42.8 (41.6-43.9)	44.0 (43.2-44.9)	36.5 (34.9-38.2)	34.5 (32.8-36.2)
800-μm	43.1 (41.8-44.3)	44.3 (43.4-45.2)	37.1 (35.4-38.9)	35.0 (33.4-36.6)
1000-μm	42.7 (41.2-44.1)	44.2 (43.3-45.1)	37.1 (35.2-39.1)	35.0 (33.3-36.6)
1200-μm	42.5 (41.0-43.9)	43.8 (42.7-44.9)	37.1 (35.1-39.1)	34.5 (32.5-36.4)
1400-μm	41.6 (40.1-43.1)	43.6 (42.5-44.8)	36.7 (34.4-38.9)	33.6 (31.4-35.7)

NoDR = no clinically observable diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

differences between capillaries and noncapillary blood vessels suggest that their responses to hyperglycemia may differ, and therefore removing noncapillary blood vessel areas from perfused density analysis in order to increase the sensitivity of detection of changes to the capillary bed makes sense.

The increase in PCD observed in the NoDR group could be attributable to new capillaries as in neovascularization, recruitment of reserve nonperfused capillary segments, or dilatation of existing capillaries. By definition, the eyes in the NoDR group did not have neovascularization, but recruitment may have contributed to a very limited extent. It is likely that dilatation of existing capillaries accounts for the bulk of the increased PCD. Capillary dilatation markedly increases volumetric retinal blood flow, by the fourth power of the lumen radius according Poiseuille's equation. This increased blood flow through the retina in patients with diabetes mellitus without diabetic retinopathy has been reported in several studies using a variety of methods.[33,47,48] Furthermore, increased blood flow secondary to capillary dilatation could produce the higher intravascular oxygen concentration in arterioles that has been demonstrated using dual-wavelength oximetry in type II diabetics without diabetic retinopathy.[49] Conversely, blood flow has been shown to decrease in NPDR, which may be owing to increased resistance from progression of capillary nonperfusion.[50,51]

Our methodology of determining PCD implies that our PCD results rely on a balance between capillary dilatation and capillary nonperfusion. Development of capillary segment nonperfusion in diabetic eyes must occur for some period of time before clinical retinopathy is evident.[31] It is likely to begin among beds of dilated capillaries, resulting in some masking of a PCD reduction in the early stages. As disease worsens, nonperfusion overrides the effect on PCD of the earlier compensatory dilatation, and thus PCD first increases and then decreases. Interestingly, although previous studies showed a decreased perfused vessel density in diabetic eyes without retinopathy, they also showed an increased vessel diameter index, which is a measure for vessel caliber.[37,41] Furthermore, as disease worsens, blood viscosity increases and the elasticity of the vessel wall decreases, both of which would theoretically lead to decreased blood flow through the retina, leading to decreased PCD.[52]

Relative tissue hypoxia is thought to be an early inciting event in the pathogenesis of diabetic microvascular disease. Direct evidence of inner retinal hypoxia in diabetic cats without retinopathy has been demonstrated using an intraretinal electrode.[53] Several mechanisms have been proposed to account for the relative retinal hypoxia. The tissue demand for oxygen is increased, thought to be largely owing to the need to accommodate increased levels of glucose. Relative hypoxia can also occur as a result of reduced oxygen extraction from blood vessels by the retinal tissues. This has been evidenced by increased venous oxygen saturation in both type 1 and 2 diabetics without diabetic retinopathy.[49,54] Decreased tissue oxygen extraction in diabetics may occur by shunting in the setting of subclinical capillary nonperfusion and thickening of capillary basement membranes. Ditzel[55] as well as Standl and Kolb[56] proposed that decreased oxygen extraction may be secondary to an increased oxygen affinity of hemoglobin in diabetics. Ditzel showed that at physiologic blood oxygen levels, hemoglobin can release up to 30% less oxygen in diabetics.[55] In the setting of relative tissue hypoxia, Kohner in 1975[57] and Yoshida and associates in 1983[58] proposed that a functional capillary dilatation precedes structural changes. Many years of research later, we now know that the retinal neurovascular unit responds to tissue hypoxia (decreased pO2) and decreased pH (from increased lactic acid levels produced by anaerobic metabolism) with a functional autoregulatory dilatation of the capillaries.[59] In fact, several studies have shown that hyperglycemia is associated with vascular dilatation and retinal hyperperfusion.[60,61] Reactive oxygen species and the inflammatory milieu in diabetes[62,63] likely contribute to functional capillary dilatation, as well, besides causing increased permeability, leukocyte stasis, and adhesion.

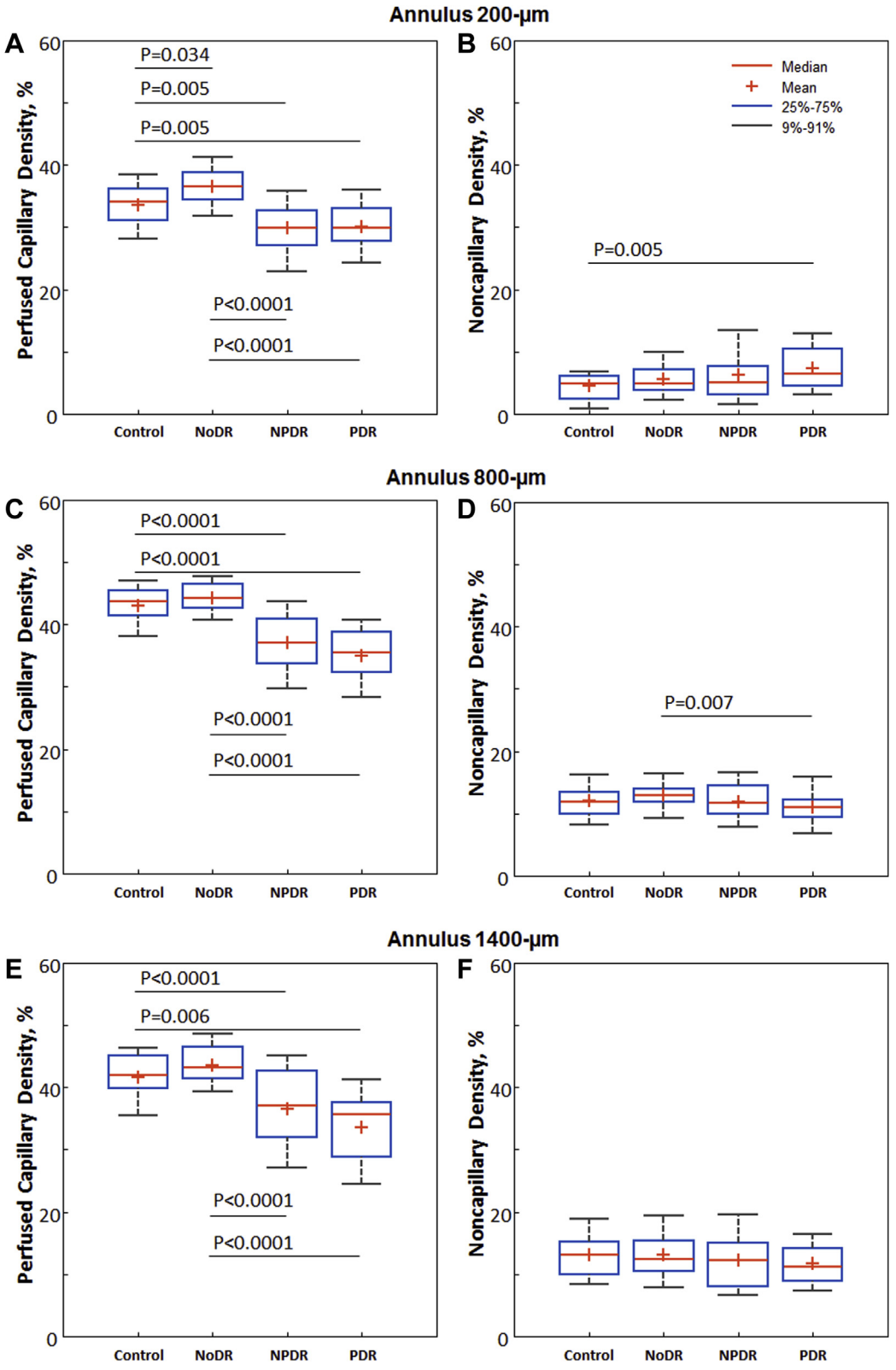

FIGURE 4. Box plots of PCD (Left column) and noncapillary blood vessel density (Right column) measured at the (A, B) 200-µm, (C, D) 800-µm, and (E, F) 1400-µm annuli. Brackets indicate statistically significant differences between corresponding study groups. NoDR = no clinically observable diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

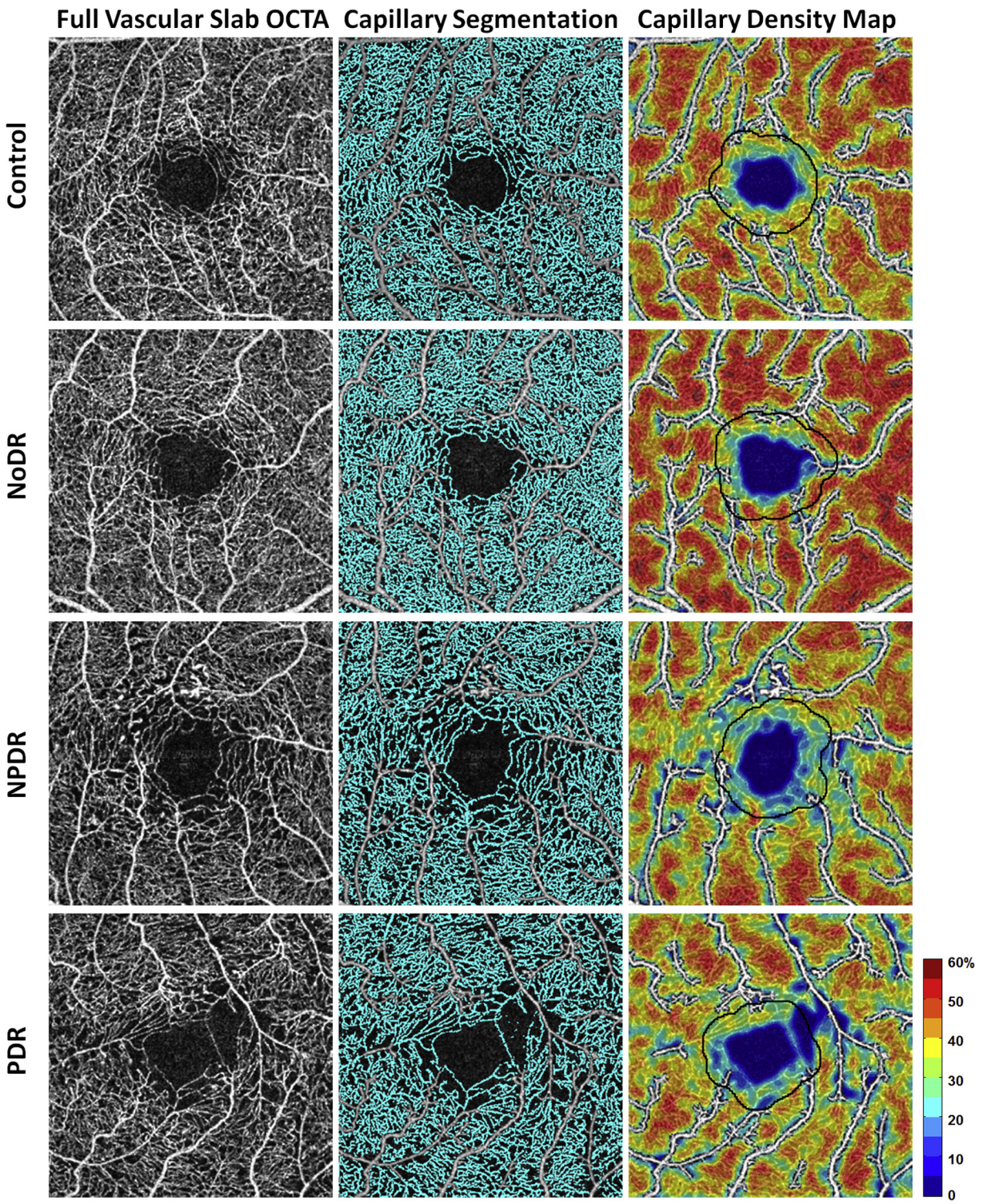

FIGURE 5. Comparison of PCD maps in a healthy control and patients with various stages of diabetic retinopathy. (Left column) Contrast-stretched full vascular slab optical coherence tomography angiography (OCTA). (Middle column) Corresponding capillary segmentation highlighted in cyan. (Right column) Corresponding PCD maps with noncapillary blood vessels indicated in white. The 200-μm annuli are delineated in all subjects for easier comparison. NoDR = no clinically observable diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

Lorenzi and associates proposed another potential mechanism for increased retinal blood flow in well-controlled type 1 diabetics without retinopathy.[64] They reported on the absence of retinal arterial constriction in response to a pressure stimulus (reclining), attributing it to a defect in the myogenic response of the smooth muscle cells of retinal arterioles in the setting of diabetes. According to their report, this was the first detectable abnormality of the retinal vessels in these subjects. Theoretically, impaired contractility of the arterioles would cause engorgement of downstream capillary beds, manifesting as an increased PCD on OCTA imaging.

A more permanent structural dilatation may occur with prolonged functional dilatation and endothelial cell proliferation in the setting of continued hypoxia.[65] Increased blood flow through the dilated retinal capillaries would lead to damage to the endothelial cells and, with concomitant pericyte loss and formation of advanced glycation end-products, would result in compromise of the microvascular wall integrity. This in turn would lead to the recognizable signs of clinical NPDR, such as capillary nonperfusion, microaneurysms, and dot-and-blot hemorrhages. Fu and associates have attempted to develop a model of this proposed sequence, to explain how expanding capillary segment nonperfusion owing to progressive hypoxia could lead to the larger areas of nonperfusion that follow in NPDR and PDR.[66]

PCD increases were highest in the innermost 200-μm annulus surrounding the FAZ, which is consistent with the proposed model of early pathogenesis of diabetic microvascular disease. The impact of relative tissue hypoxia would be expected to be most evident adjacent to the central fovea, the region of highest metabolic demand in the retina.

Limitations of this study most notably include sample size and the demographics of the study population. Patients were self-reported diabetics. Onset of diabetes and management history, as well as blood pressure, cardiovascular health, and laboratory data such as hemoglobin A1c, blood sugar levels, and lipids, were not addressed or controlled for. The permutations of these variables may also have contributed to difference in our finding compared to reports that only detected decline in PCD in their diabetes mellitus NoDR patients. A more comprehensive evaluation of these elements is planned for a future study to elucidate the impact of these factors. The contribution of insulin therapy in some of the patients in this study may have been an additional confounder. Insulin has a direct vasodilator effect on retinal arterioles via the same pathways used by capillary autoregulation—nitric oxide release in vascular endothelial cells.[67] While its impact on the capillary endothelium in early diabetic disease is complex, it has been shown that during later stages of diabetic retinopathy, endothelial cell insulin resistance contributes to capillary constriction and occlusion.[68]

The methodology of annular PCD computation is another potential source of error, since it relies on manual demarcation of the FAZ margin on the OCTA image. In this study we found that FAZ does not change significantly between the control and NoDR groups, and thus allowed for a fair comparison of annular PCD between these 2 groups. However, as the incidence of capillary nonperfusion surrounding the FAZ increases with worsening NPDR and PDR,[36] FAZ parameters change as well. Demarcating the FAZ margin on the OCTA image in the NPDR and PDR groups introduces a potential error, since it is not detecting the nonperfused occluded capillaries encircling the true structural FAZ margin. This source of error can potentially be solved by delineating the FAZ margins based on the respective en face OCT reflectance images.[69]

OCTA is a promising noninvasive imaging modality that has the potential to reveal the earliest signs of retinal diabetic microvascular disease. The upward inflection in PCD signals a "tipping point" prior to worsening disease and may serve as a useful biomarker. The clinical significance of such a phenomenon will depend on its utility for earlier detection of retinopathy and individual risk assessment in predicting the onset of the spectrum of ocular and systemic complications of diabetes. The cross-sectional design of this study was a necessary first step. Tracking the progression in a longitudinal study through the peak of compensatory engorgement into the progressive decline in perfusion seen in NPDR and PDR eyes is potentially like watching the proverbial "canary in the coal mine." Its recognition provides the rationale for more aggressive management of the diabetes, which can lead to reduction and potential reversal of ocular and systemic complications at earlier stages of the disease than is currently possible. Reversibility of retinal capillary damage has already been demonstrated in response to improvement in blood glucose management, following anti-VEGF therapy, and in response to therapeutic manipulation of factors such as Tie-2,[70] kallikrein,[71] and VAP-1.[72] Future studies should also analyze the superficial and deep parafoveal capillary networks separately, in order to determine which is more sensitive in early detection of diabetic microvascular disease in diabetic eyes without clinical retinopathy. OCTA PCD monitoring shows promise as a noninvasive detector of early diabetic retinal dysfunction, is well suited for studies of systemic pharmacologic agents targeting capillary stabilization or reperfusion,[73,74] and may radically alter future management of the disease.

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